CLAIMS

WHAT IS CLAIMED IS:

1. A chimeric protein comprising, from N-terminus to C-terminus:

a) a first peptidyl fragment consisting of an amino acid sequence that has at least 40% identity to a domain containing at least first 20 N-terminal amino acids of human growth hormone (hGH) protein, in which the percentage identity is determined over an amino acid sequence of identical size to the domain of hGH;

b) an Arg residue, or a Lys residue, or a second peptidyl fragment consisting of at least 2 amino acids in which peptidyl fragment the most C-terminal amino acid residue is an Arg or a Lys residue; and

c) a third peptidyl fragment consisting of an amino acid sequence containing more than two cysteine (Cys) residues which peptidyl fragment is not a portion of hGH protein.

2. The chimeric protein of claim 1, wherein the first peptidyl fragment consists of an amino acid sequence that has at least 60% identity to the domain of hGH protein.

3. The chimeric protein of claim 1, wherein the first peptidyl fragment is capable of being bound by an anti-hGH antibody.

4. The chimeric protein of claim 1, wherein the first peptidyl fragment consists of the amino acid sequence of SEQ ID NO:1.

5. The chimeric protein of claim 1, wherein the first peptidyl fragment consists of the amino acid sequence of SEQ ID NO:2.

6. The chimeric protein of claim 1, wherein the second peptidyl fragment consists of the amino acid sequence of SEQ ID NO:3.

7. The chimeric protein of claim 1, wherein the third peptidyl fragment is an insulin precursor.

8. The chimeric protein of claim 7, wherein the insulin precursor is of

human origin.

9. The chimeric protein of claim 8, wherein the human insulin precursor is capable of being bound by an anti-human-insulin antibody.

10. The chimeric protein of claim 8, wherein the human insulin precursor consists of the amino acid sequence of SEQ ID NO:4.

11. The chimeric protein of claim 8, wherein in the human insulin precursor, B chain and A chain of the human insulin precursor are separated by an amino acid residue or a peptidyl fragment consisting of 2 to 34 amino acid residues.

12. The chimeric protein of claim 8, wherein the human insulin precursor consists of the amino acid sequence of SEQ ID NO:5.

13. A chimeric protein consisting of the amino acid sequence of SEQ ID NO:6.

14. A chimeric protein consisting of the amino acid sequence of SEQ ID NO:7.

15. An isolated nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 1.

16. An isolated nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 13.

17. An isolated nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 14.

18. The nucleic acid of claim 15, wherein said nucleic acid is a DNA.

19. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 15.

20. An isolated nucleic acid hybridizable to the nucleotide sequence

encoding the first, second and third peptidyl fragments of the DNA of claim 18.

21. A recombinant cell containing the nucleic acid of claim 15.

22. A recombinant cell containing the nucleic acid of claim 16.

23. A recombinant cell containing the nucleic acid of claim 17.

24. A method of producing a chimeric protein comprising growing a recombinant cell containing the nucleic acid of claim 15 such that the encoded chimeric protein is expressed by the cell, and recovering the expressed chimeric protein.

25. A method of producing a chimeric protein comprising growing a recombinant cell containing the nucleic acid of claim 16 such that the encoded chimeric protein is expressed by the cell, and recovering the expressed chimeric protein.

26. A method of producing a chimeric protein comprising growing a recombinant cell containing the nucleic acid of claim 17 such that the encoded chimeric protein is expressed by the cell, and recovering the expressed chimeric protein.

27. The product of the process of claim 24.

28. The product of the process of claim 25.

29. The product of the process of claim 26.

30. A process for obtaining a correctly folded first insulin-precursor-containing chimeric protein, comprising contacting an incorrectly folded second insulin-precursor-containing chimeric protein, which said second insulin-precursor-containing chimeric protein consists of an intramolecular chaperone (IMC) like peptidyl fragment separated from the insulin precursor by one or more cleavable amino acid residues, with at least one chaotropic auxiliary agent in an aqueous medium; wherein said IMC like peptidyl fragment:

a) contains from about 20 to about 200 amino acid residues;
b) is not the insulin precursor or a portion thereof; and
c) improves the insulin precursor folding such that the yield of the correctly

folded first insulin-precursor-containing chimeric protein when the incorrectly folded second insulin-precursor-containing chimeric protein is contacted with the chaotropic auxiliary agent is higher than the yield of the correctly folded insulin precursor when the incorrectly folded insulin precursor, which does not contain said IMC like peptidyl fragment, is contacted with the same chaotropic auxiliary agent.

31. The process of claim 30, wherein the insulin precursor is of human origin.

32. The process of claim 31, wherein the human insulin precursor is capable of being bound by an anti-human-insulin antibody.

33. The process of claim 31, wherein the human insulin precursor consists of the amino acid sequence of SEQ ID NO:4.

34. The process of claim 31, wherein in the human insulin precursor, B chain and A chain of the human insulin ~~precursor~~ are separated by an amino acid residue or a peptidyl fragment consisting of 2 to 34 amino acid residues.

35. The process of claim 31, wherein the human insulin precursor consists of the amino acid sequence of SEQ ID NO:5.

36. The process of claim 30, wherein the IMC like peptidyl fragment contains higher percentage of charged amino acid residue than the insulin precursor.

37. The process of claim 30, wherein in the IMC like peptidyl fragment, the N-terminal half contains more positively charged amino acid residues than negatively charged amino acid residues and the C-terminal half contains more negatively charged amino acid residues than positively charged amino acid residues.

38. The process of claim 30, wherein the IMC like peptidyl fragment consists of an amino acid sequence that has at least 40% identity to a domain containing at least first 20 N-terminal amino acids of human growth hormone (hGH) protein, in which the percentage identity is determined over an amino acid sequence of identical size to the domain of hGH.

39. The process of claim 38, wherein the IMC like peptidyl fragment consists of an amino acid sequence that has at least 60% identity to a domain containing at least first 20 N-terminal amino acids of human growth hormone (hGH) protein.

40. The process of claim 30, wherein the IMC like peptidyl fragment is capable of being bound by an anti-hGH antibody.

41. The process of claim 38, wherein the IMC like peptidyl fragment consists of the amino acid sequence of SEQ ID NO:1.

42. The process of claim 38, wherein the IMC like peptidyl fragment consists of the amino acid sequence of SEQ ID NO:2.

43. The process of claim 30, wherein the cleavable amino acid residue is an Arg or a Lys residue.

44. The process of claim 30, wherein the cleavable amino acid residues consist of the amino acid sequence of SEQ ID NO:3.

45. The process of claim 30, wherein in the incorrectly folded second insulin-precursor-containing chimeric protein, the IMC like peptidyl fragment is located at the N-terminus of said chimeric protein.

46. The process of claim 30, wherein in the incorrectly folded second insulin-precursor-containing chimeric protein, the IMC like peptidyl fragment is located at the C-terminus of said chimeric protein.

47. The process of claim 30, wherein in the incorrectly folded second insulin-precursor-containing chimeric protein, the IMC like peptidyl fragment is located between the B chain and A chain of the insulin precursor.

48. The process of claim 30, wherein the IMC like peptidyl fragment contains one or more cleavable amino acid residues which are identical to the one or more cleavable amino acid residues that separate the IMC like peptidyl fragment and the insulin precursor in the second insulin-precursor-containing chimeric protein.

49. The process of claim 48, wherein the cleavable amino acid residue is an Arg or a Lys residue.

50. The process of claim 30, wherein the incorrectly folded second insulin-precursor-containing chimeric protein consists of the amino acid sequence of SEQ ID NO:6.

51. The process of claim 30, wherein the incorrectly folded second insulin-precursor-containing chimeric protein consists of the amino acid sequence of SEQ ID NO:7.

52. The process of claim 30, wherein the chaotropic auxiliary agent is selected from the group consisting of guanidine hydrochloride, ethylene carbonate, thiocyanate, dimethyl sulfoxide and urea.

53. The process of claim 52, wherein the chaotropic auxiliary agent is urea.

54. The process of claim 53, wherein the urea is present at a concentration from about 2.0 to about 8.0 M.

55. The process of claim 54, wherein the urea is present at a concentration from about 3.0 to about 6.0 M.

56. The process of claim 30, wherein the incorrectly folded second insulin-precursor-containing chimeric protein is contacted with at least one chaotropic auxiliary agent in an aqueous medium at a pH from about 8.0 to about 10.5 and at a concentration of the incorrectly folded second insulin-precursor-containing chimeric protein from about 0.05 to about 15.0 g per liter.

57. The process of claim 56, wherein the pH is maintained from about 9.0 to about 10.0.

58. The process of claim 56, wherein the incorrectly folded second insulin-precursor-containing chimeric protein is present from about 0.5 to about 5.0 g per

liter.

59. The process of claim 58, wherein the incorrectly folded second insulin-precursor-containing chimeric protein is present from about 2.0 to about 3.0 g per liter.

60. The process of claim 30, further comprising contacting the incorrectly folded second insulin-precursor-containing chimeric protein with a quantity of a mercaptan, which quantity yields less than 5 -SH radical of the mercaptan per cysteine residue of the incorrectly folded second insulin-precursor-containing chimeric protein.

61. The process of claim 60, wherein the incorrectly folded second insulin-precursor-containing chimeric protein is contacted with the mercaptan and the chaotropic auxiliary agent concurrently.

62. The process of claim 60, wherein the incorrectly folded second insulin-precursor-containing chimeric protein is contacted with the mercaptan and the chaotropic auxiliary agent sequentially.

63. The process of claim 60, wherein the quantity of the mercaptan yields from about 0.07 to about 1.0 -SH radical of the mercaptan per cysteine residue of the incorrectly folded second insulin-precursor-containing chimeric protein.

64. The process of claim 60, wherein the mercaptan is selected from the group consisting of dithiothreitol, dithioerythrol, 2-mercaptoethanol, cysteine, methyl thioglycolate, 3-mercapto-1,2-propanediol and 3-mercaptopropionic acid.

65. The process of claim 64, wherein the mercaptan is 2-mercaptoethanol.

66. The process of claim 30, further comprising separating the correctly folded first insulin-precursor-containing chimeric protein from the incorrectly folded second insulin-precursor-containing chimeric protein.

67. The process of claim 66, wherein the correctly folded first insulin-precursor-containing chimeric protein is separated from the incorrectly folded second

insulin-precursor-containing chimeric protein by ultrafiltration.

68. The process of claim 67, wherein the ultrafiltration is carried out at a pH from about 8.0 to about 11.0.

69. The process of claim 68, wherein the ultrafiltration is carried out at a pH from about 9.0 to about 10.0.

70. The product of the process of claim 30.

71. An assay for screening an amino acid sequence for the ability to improve folding of an insulin precursor, comprising:

(a) changing the amino acid sequence of the first peptidyl fragment of the chimeric protein of claim 1, obtaining said chimeric protein with said changes, contacting said chimeric protein with said changes with at least one chaotropic auxiliary agent in an aqueous medium under conditions and for a time sufficient such that said chimeric protein folds correctly, and measuring the folding yield of said chimeric protein with said changes;

(b) obtaining the same chimeric protein used in step (a), but without any amino acid sequence changes described in step (a), contacting the chimeric protein without any amino acid sequence changes described in step (a) with at least one chaotropic auxiliary agent in an aqueous medium under the same conditions and for a same time used in step (a), and measuring the folding yield of the chimeric protein; and

(c) comparing the folding yield of the chimeric proteins measured in step (a) and (b), respectively,

in which the yield measured in step (a) substantially equals or is greater than the yield measured in step (b) indicates that the amino acid sequence improves folding of the insulin precursor.

72. The assay of claim 71, wherein the chimeric protein consists of the amino acid sequence of SEQ ID NO:6.

73. The assay of claim 71, wherein the chimeric protein consists of the amino acid sequence of SEQ ID NO:7.

74. The assay of claim 71, wherein the chaotropic auxiliary agent is urea.

75. The assay of claim 71, further comprising contacting the chimeric protein, in step (a) and (b) respectively, with a quantity of a mercaptan, which quantity yields less than 5 -SH radical of the mercaptan per cysteine residue of the chimeric protein.

76. The process of claim 75, wherein the mercaptan is 2-mercaptoethanol.

77. The product of the assay of claim 71.